



Consommation et  
Affaires commerciales Canada

Consumer and  
Corporate Affairs Canada

Bureau des brevets

Patent Office

Ottawa, Canada  
K1A 0C9

(21) (A1)	2,086,351
(22)	1992/12/29
(43)	1993/07/10

(51) INTL.CL. <sup>5</sup> A01N-063/00; A01N-063/04

(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) **Combined Use of Chemicals and Microbials in Termite Control**

(72) Zeck, Walter M. - U.S.A. ;  
Simonet, Donald E. - Germany (Federal Republic of) ;  
Price, David A. - U.S.A. ;

(73) Miles Inc. - U.S.A. ;

(30) (US) 07/818,326 1992/01/09  
(US) 07/929,034 1992/08/12

(57) **33 Claims**

Notice: This application is as filed and may therefore contain an incomplete specification.

**Canada**

OCA 7014 (10-87) 41 7530-21-030-3364

2086351

Mo3729CIP  
CA-045-CIP

TERMITICIDE

ABSTRACT OF THE DISCLOSURE

A composition for termite treatment composed of an effective amount of (1) a chemical termiticide selected from pyrethroids, pyrazolines, nitroguanidines and nitromethylenes and (2) an entomopathogenic fungus or bacterium preferably a fungus selected from either the Conidiobolus genus or the Paecilomyces genus or the Veauveria genus or the Metarhizium genus. The chemical termiticide is applied at a site where termites have been observed or are suspected to be present. The fungus or bacterium need not be applied to the site where the termites have been observed if the fungus or bacterium is already present at that site.

Mo3729CIP

**COMBINED USE OF CHEMICALS AND  
MICROBIALS IN TERMITE CONTROL****BACKGROUND OF THE INVENTION**

5 The present invention relates to a method for  
exterminating termites and to compositions useful in such  
extermination.

A number of chemicals which kill termites at specific  
concentrations are known. Specific examples of such chemicals  
include cyfluthrin (disclosed e.g., in U.S. Patent 4,218,469),  
10 propoxur (disclosed e.g., in U.S. Patent 3,111,539),  
fenvalerate (disclosed e.g., in U.S. Patent 4,061,664),  
isofenphos (disclosed e.g., in U.S. Patent 3,621,082),  
cypermethrin (disclosed in U.S. Patent 4,024,163), and  
1-(2-chloro-5-pyridyl methyl)-2-(nitroimino)imidazolidine  
15 (disclosed, e.g. in U.S. Patent 4,742,060).

Certain microbes, specifically some entomophageous fungi  
and bacteria are known to be associated with termite colonies  
and to cause deleterious effects when certain conditions exist.  
See, for example, Ko, W.H., et al., "The Nature of Soil  
20 Pernicious to *Coptotermes Formosanus*", Journal of Invertebrate  
Pathology, Volume 39, pages 38-40 (1982).

However, each of these known termiticides, bacteria and  
fungi has characteristics which makes it commercially  
undesirable. For example, many of the known chemicals and  
25 microbes must be used at rates which are too high to be  
economical or environmentally desirable. Known termiticides  
are also often too slow acting to assure success in their  
practical application. The effectiveness of many of the known  
termiticides is dependent upon the specific environment in  
30 which they are used and may therefore be detrimentally affected  
by uncontrollable factors.

35376UM1259

2086351

It has now been found that when specific types of chemicals and fungi or bacteria selected from specific species are used in combination to treat a site infested with termites, unexpected synergism in termite control results. When used in combination, the application rates can be substantially lower than those which would be used for an individual chemical termiticide and individual microorganisms. The effects of the combined chemical and fungus or bacterium of the present invention are seen in days rather than weeks. Such combinations make it possible to achieve much higher, more predictable and more economical termite control.

The present invention is particularly advantageous in that it does not employ chlorinated hydrocarbons, the most widely used group of termite control chemicals. The present invention is based upon a new concept in termite control employing chemical agents with less residual effect than the known termiticides in combination with biological agents, and application techniques for retreatment of sites with diminishing effectiveness.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a combination of a chemical agent and a biological material which is an effective termite treatment.

It is also an object of the present invention to provide a composition which does not present the environmental problems of chlorinated hydrocarbons and which need not be used in large quantities to be effective.

It is another object of the present invention to provide a method for effectively controlling termites.

These and other objects which will be apparent to those skilled in the art are accomplished by a composition which includes (1) an effective amount of a chemical selected from nitroguanidines, nitromethylenes, pyrazolines and pyrethroids and (2a) an entomopathogenic fungus, preferably a fungus of the Conidiobolus(Entomophthora) or Metarhizium genus or a

Mo3729CIP

Paecilomyces or Beauveria or Actinomucor species or (2b) an entomopathogenic bacterium such as Serratia. The chemical is generally present in an amount such that it makes up at least 0.01 ppm in the treated medium (e.g., soil) or 1 ppm in the bait. The fungus or bacterium is generally used in an amount such that at least 1 to 100 spores per gram of treated medium are present upon contact with the termites. The optimum quantity of fungus or bacterium will depend upon the particular fungus or bacterium species involved. This treatment may be applied in the same manner used to apply other known chemical termiticides. The compositions of the present invention may also be used in bait formulations and to re-treat previously treated areas.

DETAILED DESCRIPTION OF THE PREFERRED  
EMBODIMENTS OF THE INVENTION

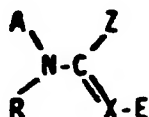
The present invention relates to a termiticide composed of (1) at least one chemical selected from (a) nitroguanidines such as 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino)imidazolidine; (b) nitromethylenes such as 1-(2-chloro-5-pyridylmethyl)-2-(nitromethylene)imidazolidine; (c) pyrazolines; and (d) pyrethroids such as cyfluthrin and either (2a) an entomopathogenic fungus such as fungus of the Conidiobolus(Entomophthora) or Metarhizium or Paecilomyces or Beauveria or Actinomucor genus or (2b) an entomopathogenic bacterium such as Serratia.

The termiticide chemicals useful in the practice of the present invention are known materials and may be made by any of the known techniques. Specific nitroguanidines and nitromethylenes and methods for making them are disclosed, for example, in the following published applications and patents: EP 464,830; EP 428,541; EP 425,978; DE 36 39 877; DE 37 12 307; US 5,034,524; EP 386,565; EP 383,091; EP 375,907; EP 364,844; JP 02.207 083; EP 315,826; EP 259,738; EP 254,859; JP 63 307,857; JP 63 287,764; EP235,725; EP 212,600; EP 192,060; EP 163,855; EP154,178; EP 136,636; US 4,948,798; EP 303,570; EP No3729CIP

302,833; US 4,918,086; EP 306,696; F.. 2,611,114; EP 183,972; EP 455,000; JP A3 279,359; JP A3,246,283; WO91/17,650; WO 91/104,965; US 5,039,686; EP 135,956; US 5,034,404; EP 471,372; EP 302,389; JP 3,220,176; Brazil 8,803,621; JP 3,246,283; JP A92/9371; and JP 3,255,072.

For example, US Patent 4,742,060 discloses that 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine may be made by reacting a solution of N-(2-chloro-5-pyridylmethyl) ethylenediamine in toluene with cyanogen bromide at room temperature. The 1-(2-chloro-5-pyridylmethyl)-2-iminoimidazolidine hydrobromide thus formed was further reacted with sulfuric acid and fuming nitric acid. The dichloromethane solvent was removed and the desired 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine was recovered.

Chemical termiticides which are suitable for use in the practice of the present invention are represented by the formula



(I)

in which

R represents hydrogen, an acyl group, a substituted acyl group, an alkyl group, a substituted alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, or a substituted heterocyclic group;

A represents either (1) a monofunctional group selected from hydrogen, an acyl group, an alkyl group, an aryl group, a substituted acyl group, a substituted alkyl group, a substituted aryl group or (2) a bifunctional group which is connected to Z;

E represents an electron withdrawing group;

No3729CIP

- X represents N or CH<sub>3</sub>; and  
Z represents either (1) a monofunctional group selected from hydrogen, an acyl group, a substituted acyl group, an alkyl group, a substituted alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, a substituted heterocyclic group, the group OR, NR<sub>2</sub>, SR or (2) a bifunctional group which is connected with either A or X.

Preferred acyl and substituted acyl groups include alkylcarbonyl, substituted alkylcarbonyl, arylcarbonyl, substituted arylcarbonyl, alkylsulfonyl, substituted alkylsulfonyl, arylsulfonyl, substituted arylsulfonyl, alkylphosphoryl, substituted alkylphosphoryl, arylphosphoryl, and substituted arylphosphoryl.

Preferred alkyl substituents include: C<sub>1</sub>-C<sub>10</sub> alkyl groups which are optionally substituted, particularly C<sub>1</sub>-C<sub>4</sub> alkyl groups which are optionally substituted and most preferably methyl, ethyl, n-propyl, isopropyl, n-butyl, secondary butyl or tertiary butyl groups.

Preferred aryl groups include phenyl and naphthyl, most preferably phenyl groups which may optionally be substituted.

Preferred heterocyclic groups include aromatic rings having up to 10 atoms on the ring with at least one N, O or S atom in the ring (particularly N). Particularly preferred heterocyclic groups are thiophenyl, furyl, thiazolyl, imidazolyl, pyridyl and benzthiazolyl.

Suitable substituents for the acyl, alkyl, aryl and heterocyclic groups described above include: alkyl groups having from 1 to 4 carbon atoms, preferably 1 or 2 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, i-butyl, and t-butyl; alkoxy groups with from 1 to 6 carbon atoms, preferably 1 or 2 carbon atoms such as methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, i-butoxy, and t-butoxy; alkylthio groups having from 1 to 4 carbon atoms, preferably 1 or 2 carbon atoms, such as methylthio, ethylthio, n-propylthio,

Mo3729C1P

1-propylthio, and n-butylthio, i-butylthio and t-butylthio; haloalkyl with from 1 to 4 carbon atoms, particularly 1 or 2 carbon atoms, and from 1 to 5, preferably 1 to 3 halogen atoms which halogen atoms may be the same or different, particularly F, Cl or Br (most preferably F), such as trifluoromethyl; hydroxyl groups; halogen such as fluoride, chloride, bromide and iodide, particularly fluoride, chloride and bromide; cyano groups; nitro groups; amino groups; monoalkyl- and dialkyl-amino groups from 1 to 4, preferably 1 or 2 carbon atoms in the alkyl group such as methylamino, methyl-ethyl-amino, n-propylamino, i-propylamino, and methyl-n-butylamine; carboxyl groups; alkoxy carbonyl groups with from 2 to 4 carbon atoms, preferably 2 or 3 carbon atoms, such as carbomethoxy and carboethoxy; sulfo groups ( $\text{SO}_3\text{H}$ ); alkylsulfonyl groups with from 1 to 4, preferably 1 or 2, carbon atoms, such as methylsulfonyl and ethylsulfonyl; and aryl sulfonyl groups with from 6 to 10 carbon atoms on the aromatic ring such as phenylsulfonyl.

In Formula I, A and Z together may form a saturated or an unsaturated heterocyclic ring with from 5 to 7, preferably 5 or 6, atoms in the ring. This ring may contain 1 or 2 heterocyclic atoms such as O, S, N and N-alkyl. If two atoms in the ring are not carbon, those two atoms may be the same (e.g., two N atoms) or different (e.g., one N and one O).

Specific examples of electron withdrawing groups which are represented by the radical E in Formula I include  $\text{NO}_2$ , CN, and halogenalkyl-carbonyl groups such as the 1,5-halogeno- $\text{C}_1\text{-C}_6$  alkyl-carbonyl groups.

In Formula I, Z and X together may form a heterocyclic ring from 5 to 7, preferably 5 or 6 atoms in the ring. The ring may contain 1 or 2 heterocyclic atoms such as O, S, N or N-alkyl which heterocyclic atoms may be the same or different. Specific examples of preferred heterocyclic rings include pyrrolidine, piperidine, piperazine, hexamethyleneimine, morpholine and N-methylpiperazine.

M53729CIP



Preferred compounds within the scope of Formula I are those in which

A and Z each represents hydrogen, an alkyl group which may be substituted, an acyl group which may be substituted, a phosphonyl group which may be substituted, or the group  $\text{NR}'\text{R}''$  in which  $\text{R}'$  and  $\text{R}''$  each represents hydrogen or an alkyl group, or together represent an optionally substituted five or six membered ring which may have nitrogen or sulfur as one of its members.

E represents an electron withdrawing group such as  $\text{NO}_2$ ,  $\text{CN}$  or  $\text{COCF}_3$ .

X represents  $\text{N}$  or  $\text{CH}$ ,

and

R represents hydrogen, a pyridyl group which may be substituted, a pyridylalkyl group, a thiazolylalkyl group, an alkyl thioalkyl group, or a five or six membered ring having oxygen, nitrogen or sulfur as one of its members which ring may be substituted with one or more halogen, alkyl, haloalkyl or  $\text{NO}_2$  groups.

Particularly preferred compounds include those represented by formula I in which

Z represents  $\text{NH}$ ,  $\text{CH}$  or  $\text{NR}'\text{R}''$  in which at least one of  $\text{R}'$  and  $\text{R}''$  is an alkyl group,

A represents an alkyl group,

A and Z together represent a nitrogen-containing 5 or 6 membered ring optionally substituted with a thioalkyl group or a sulfur containing 5 or 6 membered ring optionally substituted with a thioalkyl group,

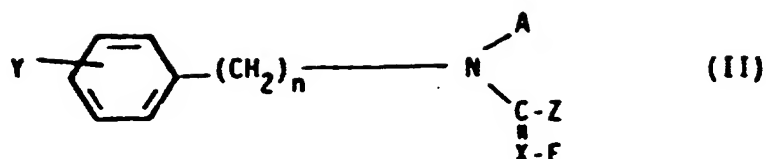
E represents  $\text{CN}$  or  $\text{NO}_2$ ,

X represents  $\text{N}$  or  $\text{CH}$ , and

R represents a 5 or 6 membered ring having  $\text{O}$ ,  $\text{N}$  or  $\text{S}$  as at least one member of the ring which ring is optionally substituted with a halogen or alkyl group.

and those represented by the formulae

**No3729C1P**



5

in which

n represents 1 or 2.

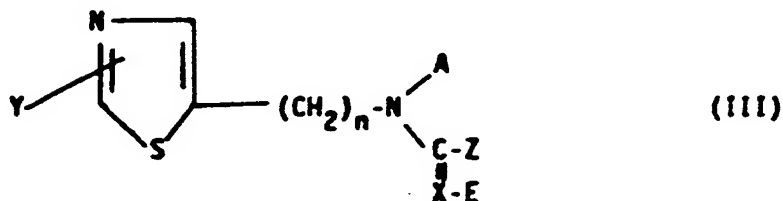
Y represents any of the substituents described above as being suitable substituents for the acyl, alkyl, aryl and heterocyclic groups in formula I, preferably a

10

A, Z, X and E have the same meaning as in Formula I,

and

15



20

in which A, Z, X, E, Y and n have the same meaning as in Formulae I and II.

Specific examples of nitroguanidines and nitromethylenes which may be used in the present invention include:

25

3-(2-chloro-5-pyridylmethyl)-2-(nitroimino)-thiazolidine;

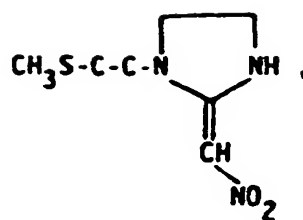
1-(2-chloro-5-pyridylmethyl)-2-(nitroimino)-imidazolidine;

1-(2-chloro-5-pyridylmethyl)-2-(nitromethylene) imidazolidine;

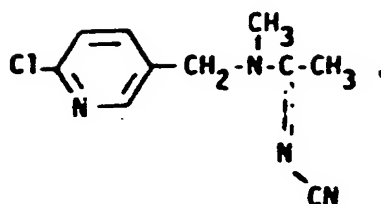
30

Mo3729CIP

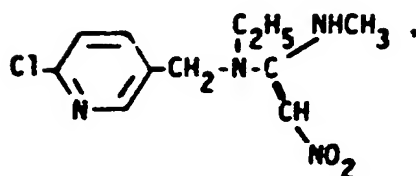
5



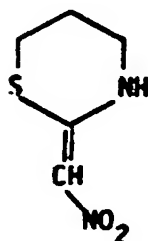
10



15



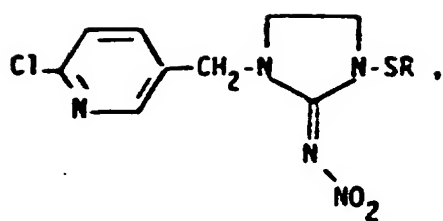
20



25

30

Mo3729CIP

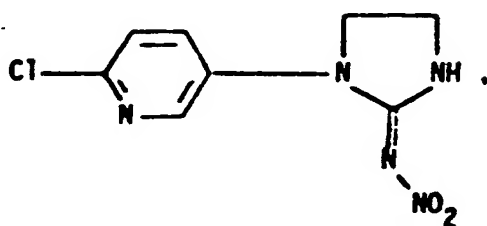


5

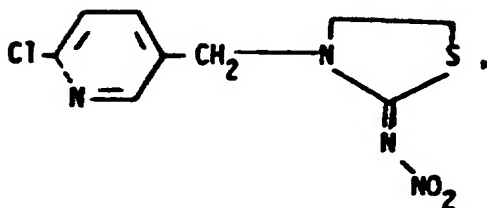
in which

R represents hydrogen, an alkyl substituted alkyl, aryl or substituted aryl group,

10

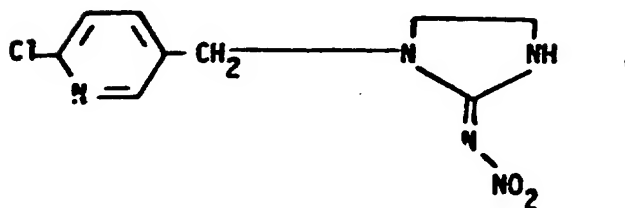


15



20

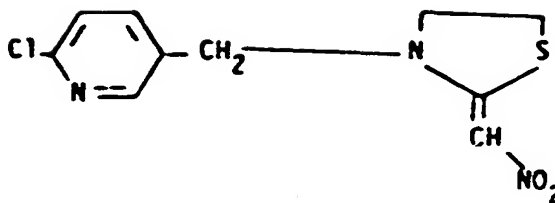
25



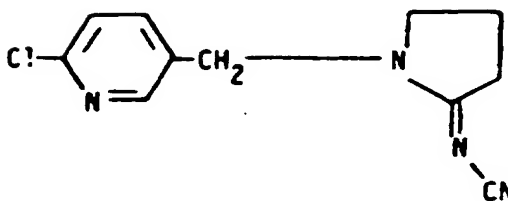
30

Mo3729CIP

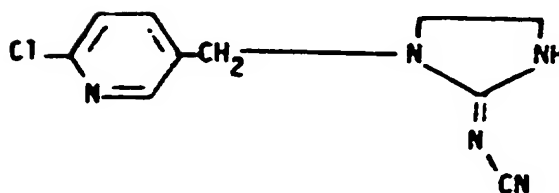
5



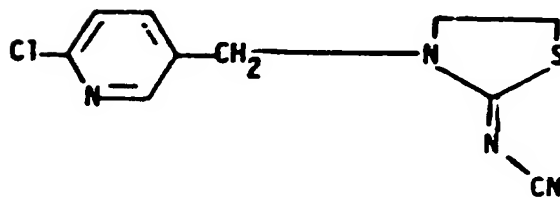
10



15

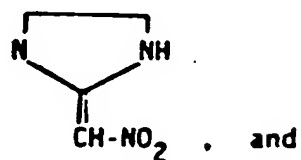


20



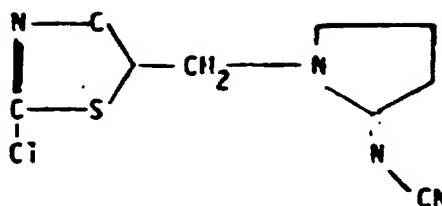
25

30



and

Mo3729CIP



5

Particularly preferred nitroguanidines and nitromethylenes are 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino)imidazolidine.

10 When used in the form of a bait, a nitroguanidine or nitromethylene is generally used in a quantity such that it represents at least 0.0001% by weight, preferably from about 0.001 to about 10.0% by weight, and most preferably from about 0.01 to about 1.0% by weight of the total bait components.

15 When the nitroguanidine or nitromethylene is incorporated directly into soil or applied directly to a surface being treated, it is generally used in an amount such that at least 0.01 ppm (parts per million), preferably from about 0.1 ppm to about 1000 ppm and most preferably from about 1 ppm to about

20 300 ppm are present in the soil or on the surface being treated.

Pyrethroids which may be used in the compositions of the present invention are known. Both naturally occurring and synthetic pyrethroids are suitable. Examples of suitable

25 pyrethroids include: pyrethrins, cinerins, jasmolins, allethrin [2-methyl-4-oxo-3-(2-propenyl)-2-cyclo-penten-1-yl-2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate], bioallethrin [D-trans-allethrin], barthrin [6-chloro-1,3-benzodioxol-5-yl)methyl 2,2-dimethyl-3-(2-methyl-1-propenyl)-

30 cyclopropanecarboxylate], tetramethrin [(1,3,4,5,6,7-hexahydro-1,3-dioxo-2H-isoindol-2-yl)methyl 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate], furamethrin [(5-(2-propenyl)-2-uranyl)methyl 2,2-dimethyl-3-(2-methyl-1-

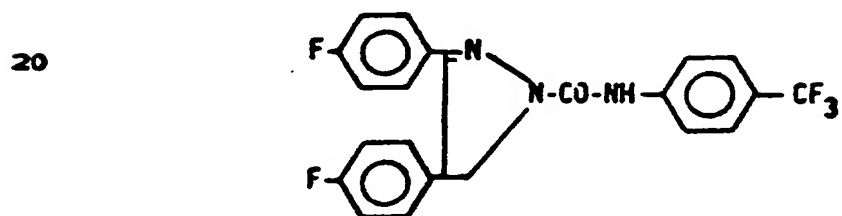
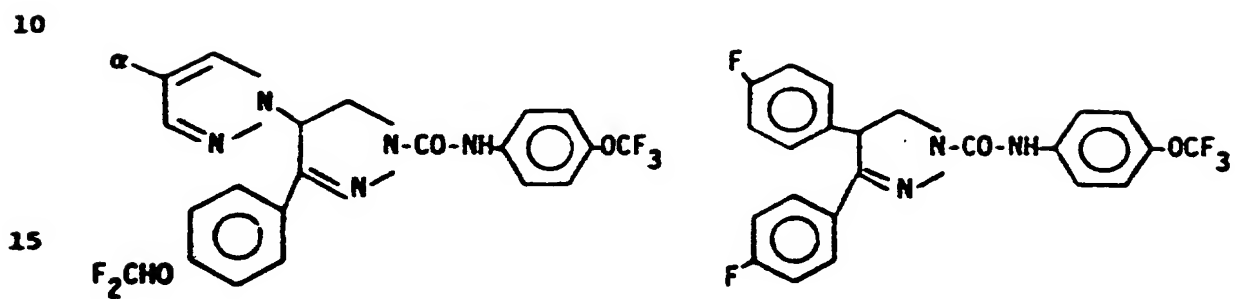
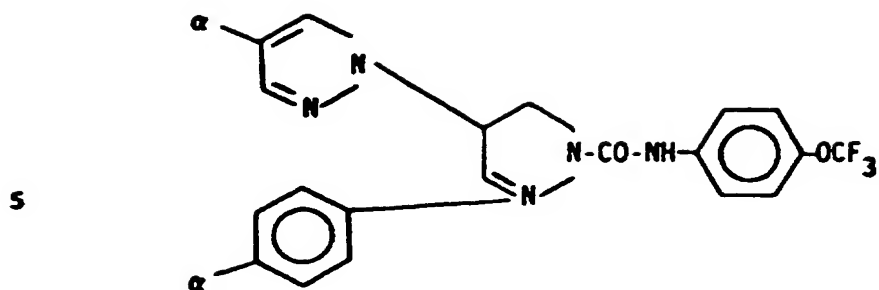
Mo3729CIP

propenyl)cyclopropanecarboxylate], resmethrin [[5-(phenyl-  
methyl)-3-furanyl]methyl 2,2-dimethyl-3-(2-methyl-1-  
propenyl)cyclopropanecarboxylate], bioethanomethrin  
[[5-(phenylmethyl)-3-furanyl]methyl 3-(cyclopentylidene-  
methyl)-2,2-dimethyl-cyclopropanecarboxylate], phenothrin  
[(3-phenoxyphenyl)methyl 2,2-dimethyl-3-(2-methyl-1-propenyl)-  
cyclopropanecarboxylate], fenpropanate [cyano(3-phenoxyphenyl)-  
methyl 2,2,3,3-tetramethylcyclopropanecarboxylate], permethrin  
[(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethyl-  
cyclopropanecarboxylate], cyfluthrin [cyano(4-fluoro-3-phenoxy-  
phenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-  
carboxylate], cypermethrin [cyano(3-phenoxyphenyl)-methyl  
3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane-carboxylate],  
decamethrin [cyano(3-phenoxyphenyl)methyl 3-(2,2-dibromo-  
ethenyl)-2,2-dimethylcyclopropanecarboxylate], fenvalerate  
[cyano(3-phenoxyphenyl)methyl 4-chloro- $\alpha$ -(1-methylethyl)-  
benzeneacetate], and cyhalothrin [cyano(3-phenoxy-phenyl)-  
methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-  
cyclopropanecarboxylate]. Particularly preferred pyrethroids  
are cyfluthrin and fenvalerate.

The pyrethroid is generally used in bait compositions in a  
quantity such that at least 0.0001% by weight, preferably from  
about 0.01 to about 10.0% by weight, and most preferably from  
about 0.01 to about 1.0% by weight of the bait composition is  
pyrethroid. Where the pyrethroid is added directly to soil or  
applied directly to a surface to be treated, the pyrethroid is  
generally used in a quantity such that at least 0.01 ppm,  
preferably from about 0.1 to about 1000 ppm, and most  
preferably from about 1.0 to about 300 ppm are present.

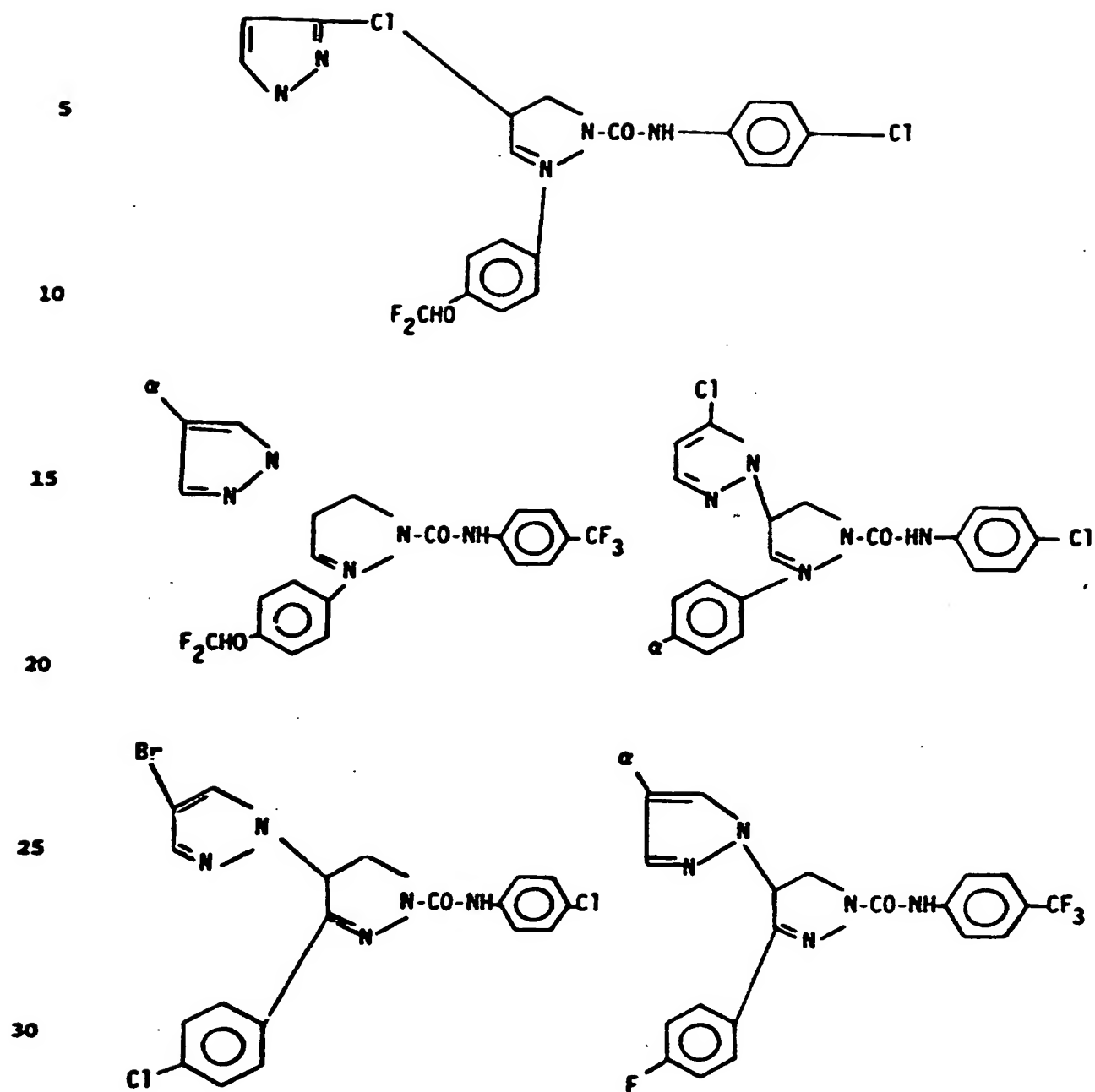
Any of the known pyrazolines may also be employed in the  
termite treatment of the present invention. Such pyrazolines  
are, for example, disclosed in published European Patent  
Application 0,438,690. Examples of suitable pyrazolines  
include those represented by the formulae:

Mo3729CIP

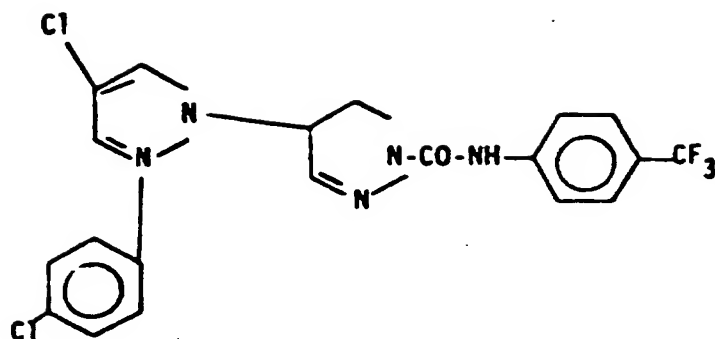


Mo3729C1P





No3729CIP



in which  $\alpha$  represents a halogen atom, an alkyl group, a halogen group or a nitro group.

The pyrazoline is generally used in bait compositions in a quantity such that at least 0.0001% by weight, preferably from about 0.001 to about 10.0% by weight, and most preferably from about 0.01 to about 1.0% by weight of the bait composition is pyrazoline. Where the pyrazoline is added directly to the soil or applied directly to the surface to be treated, the pyrazoline is used in a quantity such that at least about 0.01 ppm, preferably from about 0.1 to about 1000 ppm, and most preferably from about 1.0 to about 300 ppm are present.

The fungi employed in the termite treatment of the present invention occur naturally in soil and may be readily isolated therefrom. Species of the genus Conidiobolus (Entomophthora) useful in the termiticides of the present invention include: Conidiobolus coronatus, Conidiobolus virulenta, and Conidiobolus obscura. Conidiobolus coronatus is particularly preferred.

Species of the genus Metarhizium useful in the present invention occur naturally in soil and may be readily isolated therefrom. Various strains of Metarhizium anisopliae are useful in the present invention. The strains F 52 (BIO 1020, DSM Number 3884) and the MADA strain (received from the University of Florida)(CBS Number 326, Baarn, Netherlands) of Metarhizium anisopliae are most preferred.

Mo3729CIP

Species of the genus Paecilomyces useful in the termiticides of the present invention include Paecilomyces farinosus which naturally occurs in soil and may be readily isolated from soil or from diseased and sporulating termites by methods known to those in the art.

A preferred species of the Beauveria genus is Beauveria bassiana which may also occur naturally in soil and may be readily isolated from soil or from diseased and sporulating termites by methods known to those skilled in the art.

The Actinomucor species is also useful in the practice of the present invention.

Among the bacteria useful in the present invention are those of the Serratia species which occur naturally in soil and may be readily isolated from soil or from diseased and sporulating termites by methods known to those skilled in the art.

The fungus or bacterium should generally be present in an amount and form such that at least  $10^1$ - $10^2$  spores, preferably  $10^3$ - $10^5$  spores per grams of media are present. The optimum amount will, of course, depend on the species used.

The termite treatment of the present invention may be applied in the form of a powder, solution, suspension, emulsion, foam, paste, granules, aerosols, natural and synthetic materials impregnated with active compound and fungus and very fine capsules in polymeric substances. When in powder or granular form, the fungus may be added to the solid nitroguanidine, nitromethylene, pyrazoline or pyrethroid formulation in an appropriate amount. Other known additives for termiticides such as extenders, attractants, feeding stimulants, pheromones, may optionally be included in the final composition. Examples of suitable powder vehicles include clay, talc, lime and pyrophyllite.

The termiticide of the present invention may also be used in a variety of liquid forms. Suitable liquid vehicles for the termiticide include water and inert solvents. Other additives

Mo3729CIP

which are commonly used in liquid insecticide formulations, e.g., emulsifiers may also be included in liquid termiticide formulations of the present invention. Liquid formulations of the chemical and/or fungal agent may also include lignins, hydrocelluloses, bentonites, pectins, or any other material which causes the formulation to solidify after application.

The chemical compound and the fungus or bacterium could be sequentially applied to the medium. When this technique is used, either the chemical compound or the fungus can be applied first. The interval between application of the chemical and fungus may be as short as a few minutes or as long as a few days or even a few weeks. If an appropriate strain of the fungus or bacterium is already present as a naturally occurring material with the necessary spore titer in the medium to be treated, addition of fungus or bacterium is unnecessary and only the chemical compound need be applied. These treatments may also be made repeatedly in regular or irregular intervals to assure long-lasting effect.

The termiticides of the present invention are effective against all types of termites but have been found to be particularly effective against the subterranean termite Reticulitermes flavipes and the formosan termite Coptotermes formosanus.

The data presented below on the effects of the combined applications of chemical and biological agents reveal an impressive degree of synergism. It would take application rates higher by several powers of ten if either of these agents were used alone for termite control to achieve termite control comparable to that obtained by the combined application of the present invention.

Having thus described our invention, the following examples are given as being illustrative thereof. All parts and percentages given in these examples are parts by weight and percentages by weight, unless otherwise indicated.

Mo3729CIP

### EXAMPLES

Examples 1 through 5 demonstrate the high degree of termite extermination in short periods of time achieved by the use of a chemical termiticide in combination with a fungus as compared to extermination with chemical termiticide alone or fungus alone. Examples 6 and 7 illustrate the interaction of Conidiobolus coronatus and various chemical termiticides in termite extermination. Examples 8, 9 and 10 illustrate the interaction of 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino)-imidazolidine and two fungal pathogens of the Metarhizium genus and the Conidiobolus genus in quantitative terms. Example 11 illustrates the synergy of 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino)imidazolidine with three additional fungal species and one species of bacterium. Example 12 illustrates the synergy between microbes in non-sterile soil and various nitromethylenes or nitroguanidines.

#### Example 1

100 g of sterile soil were used in each of the samples described in Table 1. The control sample A was not treated with nitroguanidine, nitromethylene, pyrethroid, pyrazoline or fungus. 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino)-imidazolidine was added in various concentrations (indicated in Table 1) directly to the soil in samples B, C, D, E and F. No fungus was added to the soil in these samples. Filter paper discs which had been soaked in a solution of 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino)-imidazolidine having the concentration indicated in Table 1 were placed on top of the soil in samples G, H and I. No fungus was present in soil samples G, H or I. No nitro-guanidine was added to soil sample J but soil from a container used in a previous test in which the fungus Conidiobolus coronatus had shown up naturally was included in sample J. In soil sample K, soil from a previous test in which the fungus Conidiobolus coronatus had shown up naturally was used but in addition, the termites were

Mo3729CIP

fed with filter paper that was soaked with 0.01% of the nitroguanidine.

1 gram of subterranean termites Reticulitermes flavipes was then added to each of the soil samples A through K. The observations made over the next 18 days are summarized in Table 1.

It is evident from the data in Table 1 that 1 ppm nitroguanidine must be present in soil in order to achieve significant effect upon the termites and an eventual (after 18 days exposure) 100% mortality. Use of higher rates (e.g. 10 to 100 ppm) of nitroguanidine in the soil did not significantly change this result.

When the nitroguanidine was used in the form of a bait (i.e. on treated filter paper), smaller amounts of nitroguanidine (i.e. 0.001%; 0.003%; and 0.010%) were as effective as the larger amounts used to treat the soil.

The data in Table 1 also show that when only fungus was applied to the soil (i.e., no nitroguanidine was used), the results were the same as those observed in the untreated control. That is, intense tunneling and location at the bottom of the sample container were observed. However, when a filter paper disc having 0.01% nitroguanidine present thereon was placed on soil in which the fungus was present, 100% of the termites in the sample were dead within two days.

TABLE 1

## EFFECTS OF 1-(2-CHLORO-5-PYRIDYMETHYL)-2-(NITROIMINO)IMIDAZOLIDINE ON TERMITES

SAMPLE	TREATMENT	PPM/% NITROGUANIDINE	OBSERVATIONS (18 DAYS)
A	CONTROL	-----	intense tunneling, termites all healthy and on bottom
B	Soil	100 ppm	termites disoriented, 80-90% on the surface, not feeding, after 14 days gradually starve to death
C	Soil	10 ppm	same as sample B
D	Soil	1 ppm	same as sample B
E	Soil	0.1 ppm	partially disoriented, partially tunneling
F	Soil	0.01 ppm	same as sample A
G	Filter paper	0.01%	same as sample B
H	Filter paper	0.003%	same as sample B
I	Filter paper	0.001%	same as sample B
J	Fungus only in soil	----	same as sample A
K	Fungus in soil plus nitro- guanidine in Filter paper	0.01%	after 2 days, 100% dead, completely grown over by mycelium

No 3729CIP

**EXAMPLE 2**

The procedure of Example 1 was repeated using both sterile soil samples and soil samples in which Conidiobolus coronatus was added in the form of 1 drop of crude spore suspension.

5 This crude spore suspension was obtained by rinsing spores from one petri dish culture with 50 cc sterile water. 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine in various concentrations (indicated in Table 2) was added directly to the soil. The results of these tests are reported in Table 2.

10 The data presented in Table 2 show that change in termite behavior in the sterile soil samples was first observed at the 1 ppm nitroguanidine level. However, 100 ppm nitroguanidine were necessary to cause substantial mortality after 6 days. In the soil inoculated with the fungus, substantial mortality  
15 resulted at the 1 ppm level, with total mortality of the termites achieved at 3 ppm. Without a nitroguanidine treatment, termites in the soil inoculated with fungus behaved like those in the untreated check.



2086351

**TABLE 2**

**% TERMITE MORTALITY AFTER 6 DAYS**

SOIL TREATMENT WITH NITROGUANIDINE IN PPM	% TERMITE MORTALITY AFTER 6 DAYS	
	IN STERILE SOIL	SOIL INOCULATED W/ CONIDILOBOLUS COR.
0	0	0
0.1	0	0
0.3	0	25+
1.0	0+	75++
3.0	0	100
10.0	25++	100
100.0	80+++	100

+ MANY TERMITES DISORIENTED  
++ MOST TERMITES SEVERELY AFFECTED  
+++ ALL TERMITES SEVERELY AFFECTED

Mo3729C1P

**EXAMPLE 3**

100 grams of soil were used in each of the samples described in Table 3. Half of the soil samples were made with sterile soil. The other half were made up of sterile soil which had been inoculated with one drop per sample of crude spore suspension of Conidiobolus coronatus. The crude spore suspension was obtained by rinsing spores from a petri dish culture with 50 cc of sterile water. Filter paper discs were saturated with solutions of 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine having the concentration indicated in Table 3. 1 gram of subterranean termites Reticulitermes flavipes was added directly to each sample. The termites in each sample were then observed for a period of 6 days. The termite mortality for each sample is given in Table 3.

Results: Without any nitroguanidine, the termites did not show any response to the soil inoculation with Conidiobolus coronatus during the 6 day observation period. When treated filter paper baits were added to the sterile soil without the fungus, first symptoms of behavioral change showed up in the 0.001% treatment, but mortality remained low (8%) even at rates 100 times higher (0.1%). However, in the fungus inoculated soil, mortality was 95% at the 0.001% nitroguanidine level after 6 days.

TABLE 3

NITROGUANIDINE TREATMENT OF FILTER DISC BAITS IN %	% TERMITE MORTALITY AFTER 6 DAYS	
	IN STERILE SOIL	SOIL INOCULATED W/ <u>CONIDIOPHOLUS COR.</u>
0	0	0
0.00001	0	0
0.00003		0
0.0001	0	0(+)
0.0003		50+
0.001	2+	95+++
0.003		100
0.01	5++	100
0.1	8+++	100

(+) SOME REDUCTION OF ACTIVITY  
 + MANY TERMITES DISORIENTED  
 ++ MOST TERMITES AFFECTED  
 +++ ALL TERMITES SEVERELY AFFECTED

No 3729CIP

**EXAMPLE 4**

The effect of exposing termites directly to actively growing fungus in the presence and absence of 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine was studied. In these experiments, an agar plate with no fungus and no nitroguanidine was used as the control. One sample was an agar plate with a filter paper disk treated with 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine (0.01% active ingredient). A third agar plate was inoculated with Conidiobolus coronatus and a fourth agar plate was inoculated with the Conidiobolus coronatus fungus and treated with a filter paper disk containing 0.01% of the nitroguanidine. Termites (Reticulitermes flavipes) were added to each agar plate and observed for 4 days. The results of these tests are reported in Table 4.

In the non-inoculated Control sample, normal behavior of the termites with immediate extensive tunneling took place. No mortality was observed after 4 days. In the plate treated only with fungus, 25% mortality was observed after 4 days. In the plate treated with with nitroguanidine bait only, the termites exhibited severe intoxication and tunneling was not present. 100% mortality occurred within 1 day on the fungus plus nitroguanidine treated agar and massive sporulation occurred within 4 days on the dead termites.

2086351

TABLE 4

OBSERVATIONS AT DAYS AFTER INFESTING AGAR PLATES WITH TERMITES:			
TREATMENTS	PERCENT MORTALITY		
	1 DAY	2 DAYS	4 DAYS
AGAR PLATE ONLY	0	0	0
AGAR PLATE ONLY + nitroguanidine Bait (0.01% AI)	0*	0*	20*
FUNGAL PATHOGEN ON AGAR	0	5	25
FUNGAL PATHOGEN ON AGAR + nitroguanidine BAIT (0.01% AI)	100%		

\* Termites intoxicated.

Ms3729CIP

**EXAMPLE 5**

4 kilograms of sandy soil were sterilized by means of an autoclave. The sterilized soil was then divided into two equal portions. In one portion of the soil, the moisture content was  
5 adjusted to 10% by adding 10 ml of distilled water to each 100 grams of soil. The second portion of sterilized soil was inoculated with a crude slurry of Conidiobolus coronatus spores and mycelium. This slurry was made by scraping fungal growth from agar cultures on petri dishes into 50 ml of distilled  
10 water. The slurry was then added to the sterilized soil at a rate of 10 ml per 100 gram of soil. Each of the 2 kg batches of soil was then distributed in 20 plastic cups so that 100 g of soil were present in each cup. Ten cups of each batch received filter paper disks which had been treated with 0.01%  
15 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine. The remaining samples received filter paper disks which had been soaked in distilled water. Approximately 1 gram of live Reticulitermes flavipes was added to each sample. Observations of mortality, activity and fungal growth were made every few  
20 days for a week. The results of these tests are reported in Table 5.

Results: As expected, in the controls the termites behaved normally over the 7 day observation period. The same was true in the "fungus only" treatment with the exception that  
25 20% final mortality was observed. The same degree of mortality resulted from the "chemical only" treatment, although intoxicative effects were observed from the early stages of the study. The "fungus plus chemical" treatment resulted in 100% mortality within 2 days.

30

2086351

**TABLE 5**  
**FUNGAL PATHOGEN INTERACTION**  
**ON STERILIZED SOIL (KOCH'S POSTULATES)**  
**% MORTALITY**

TREATMENT	2 DAYS	4 DAYS	7 DAYS
CONTROL (NO FUNGUS, NO CHEMICAL)	0	0	0
FUNGUS ONLY	0	10	20
CHEMICAL ONLY	0*	0**	20**
FUNGUS + CHEMICAL 2	100	100	100
1 <i>Conidiobolus coronatus</i>			
2 1-(2-chloro-5-pyridylmethyl)-2-nitroimino imidazolidine			
3 mycelium/spores evident on corpses and walls of cups			
4 termites decomposed, sporulation complete			
* termites moved to surface			
** termites were lethargic, inactive			

Mo3729CIP

**EXAMPLE 6**

The interaction between the fungus Conidiobolus coronatus and various known termiticides (listed in Table 6) was studied. In these tests, 100 gram soil samples were used. Conidiobolus coronatus was added to half of the soil samples according to the procedure explained in Example 2. No fungus was added to the other half of the samples. The chemical termiticide was then added to each of the samples (i.e., both those which had been inoculated with fungus and those which had not been inoculated with fungus) in an amount sufficient to reach the rate reported in Table 6. 1 g of the subterranean termite Reticulitermes flavipes was then added to each sample. The samples were then observed over a period of 7 days. The observations made are reported in Table 6.

The carbamates and the organophosphates used in this test had little effect on the termites or they showed no difference in response between fungus-free and fungus-added soil. In contrast, the pyrethroid used (cyfluthrin) resulted in no kill without the fungus, but 100% mortality when the fungus was added to the soil.

No3729CIP



TABLE 6

INTERACTION IN TERMITE ACTIVITY BETWEEN CONIDIOTROPHUS  
CORONATUS AND VARIOUS TYPES OF CHEMICALS

Compound	Rate in soil ppm	Effect and % termite mortality after					
		2 d		4 d		7 d	
		no fungus	fungus	no fungus	fungus	no fungus	fungus
Carbamate <sup>2</sup>	1.0	0	0	0	0	0	0
Synth. Pyrethroid <sup>3</sup>	1.0	0**	95	0**	100 <sup>1</sup>	0**	100
	0.3	0*	10	0*	95	0	100
Org. Phosphate <sup>4</sup>	1.0	0*	0*	0	0	0	0
	0.3	0	0	0	0	0	0
Phostebupirin <sup>5</sup>	0.3	0	0	20	60	98	100
	0.1	0	0	0	0	0	0
	0.03	0	0	0	0	0	0

\* : Slight behavioral changes

\*\* : Severe intoxication

1 : Mycelium/Spores evident on corpses

2 : Propoxur

3 : Cyfluthrin

4 : Isofenphos

5 : O-[2-(1,1-dimethylethyl)5-pyrimidinyl]-O-ethyl-O-(1-methylethyl)-thiophosphoric acid ester

No 3729C19

**EXAMPLE 7**

The procedure of Example 6 was repeated using only pyrethroid termiticides. The results are reported in Table 7.

Results: Each of the three pyrethroids used (cyfluthrin, fenvalerate and cypermethrin) showed significant differences in activity between fungus-free and fungus-inoculated soil.

Cyfluthrin was the most active compound and provided at least 10 times better termite control in the fungus-added soil than the fungus-free soil at all three observation dates.

No3729C1P

TABLE 7

INTERACTION IN TERMITE ACTIVITY BETWEEN CONIDIOPHOLUS  
CORONATUS AND VARIOUS SYNTHETIC PYRETHROIDS

Compound	Rate in soil ppm	Effect and % termite mortality after					
		2 d			4 d		
		no fungus	fungus	no fungus	fungus	no fungus	fungus
Cyfluthrin	3.0	60	100 <sup>1)</sup>	100	100 <sup>1)</sup>	100	100 <sup>1)</sup>
	1.0	0	100 <sup>1)</sup>	15*	100 <sup>1)</sup>	15	100 <sup>1)</sup>
	0.3	0	60	0*	100 <sup>1)</sup>	0*	100 <sup>1)</sup>
Fenvalerate	10.0	80	100 <sup>1)</sup>	95	100 <sup>1)</sup>	95	100 <sup>1)</sup>
	3.0	0*	95	0*	100 <sup>1)</sup>	0*	100 <sup>1)</sup>
	1.0	0*	40	0*	60 <sup>1)</sup>	0	93 <sup>1)</sup>
Cypermethrin	3.0	80	98 <sup>1)</sup>	95	100 <sup>1)</sup>	95	100 <sup>1)</sup>
	1.0	0	60	0	100	0	100
	0.3	0	10	0	60	0	100

\* : Slight behavioral changes

1) : Mycelium/spores evident on corpses

No3729CIP

**EXAMPLE 8**

The following scale was used to evaluate the results of the tests reported in this Example and in Example 10.

5	RATING	% MORTALITY	SUBJECTIVE EVALUATION
	1	98-100	Excellent
	2	90-97	Very Good
	3	80-89	Good
	4	65-79	Satisfactory
10	5	45-64	Unsatisfactory
	6	30-44	Unsatisfactory
	7	20-29	Poor
	8	3-19	Poor
	9	0-2	No Effect

15

100 gram soil samples were used in this test. Some of the samples were injected with spores of Conidiobolus coronatus and some of the samples were injected with spores of Metarhizium anisopliae (MADA strain, CBS Number 326). The number of spores injected for specific samples are recorded in Tables 8A and 8B. These samples were then treated with filter paper disks which had been soaked in solutions of 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine having varying concentrations. The concentration of the solution used to prepare the disk for specific samples is reported in Tables 8A and 8B. 1 gram of the termites Reticulitermes flavipes was then added to each of the samples. The samples were then rated using the above-given scale after 7 days. The results are reported in Tables 8A and 8B. They show that for Conidiobolus by itself (Table 8A), spore densities of  $10^5$  per gram of soil have little effect on the termites. However, in soil treated with only a 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino)-imidazolidine bait at concentrations which are normally ineffective, 100% mortality resulted at spore concentrations in the soil as low as  $10^1$ . That means spore densities at least 4 powers of ten less were

35

Mo3729CIP

2086351

required when nitroguanidine treated baits were also added to  
the soil sample. When Metarhizium was added to the soil  
samples (Table 8B), spore densities could be several powers of  
ten lower where nitroguanidine treated baits were also added to  
the soil sample.

5

10

15

20

25

30

Mo3729C1P

TABLE 8A

Rates of Interaction of Nitroguanidine Bait Applications  
and Various Fungal Pathogens on Termites Activity

% Nitroguanidine in Dipping Solution	No. spores of <i>Conidiobolus Coronatus</i> per gram of Soil/Rating					
	0	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>
0	9	-	-	9	8	7
0.0001	9	-	①	①	②	①
0.001	9	-	①	①	①	①
0.01	8	①	①	①	①	①

○ Indicates strong expression of synergy

2086351

TABLE BB

No. of Spores of Metarhizium Anisopliae-  
MADA Strain per gram of soil/Rating

Nitroguanidine in Dipping Solution	10 <sup>5</sup>		10 <sup>7</sup>	
	0	9	5	6
0	9	9	5	6
0.0001	9	9	5	6
0.001	9	9	5	6
0.01	8	9	5	6

○ Indicates strong expression of synergy

No3729CIP

**EXAMPLE 9**

The procedure of Example 8 was repeated using two different strains of Metarhizium anisopliae-- the MADA strain (CBS Number 326) and the BIO 1020 strain (DSM Number 3884) and Conidiobolus coronatus. However, instead of using various concentrations of the nitroguanidine, the filter paper discs were soaked in either distilled water or a 0.001% solution of 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine. The results observed over a 10 day period are reported in Table 9.



2086351

TABLE 3  
Interaction in Termite Activity Between Nitroquandine Salt  
Application and Various Fungal Pathogens (Strains in Sterile Soil)

No3729CIP

No of Spores per gram of Soil	% Termite Mortality After					
	3 days			6 days		
	Water	Nitroquandine	0.001% Nitroquandine	Water	Nitroquandine	0.001% Nitroquandine
<b>Meterhizium Anisopliae</b>						
$10^7$	17	(92)		99	(100)	100
$10^6$	0	(85)		3	(100)	(100)
$10^5$	-	2		3	(96)	(99)
$10^4$	-	4		0	(75)	(92)
$10^3$	-	-		0	43	56
<b>Meterhizium anisopliae</b>						
$10^7$	60	(97)		99	98	100
$10^6$	0	0		1	(90)	(97)
$10^5$	-	4		1	(53)	(85)
$10^4$	-	0		0	(72)	(95)
$10^3$	-	-		0	(72)	(97)
<b>Conidiobolus coronatus</b>						
$10^4$	0	(90)		1	(99)	(100)
$10^3$	1	12		1	(99)	(99)
$10^2$	0	0		0	7	62
$10^1$	-	2		0	38	60
$10^0$	-	0		0	12	37
<b>Control</b>	0	15		0	23	57

○ Indicates strong expression of synergy

**EXAMPLE 10**

The procedure of Example 8 was repeated using Metarhizium  
anisopliae (MADA strain, CBS Number 326) and  
1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine in  
the quantities indicated in Table 10. The results observed  
after 5 days are reported in Table 10 using the same scale as  
was recited in Example 8.

The results shown in Examples 9 and 10 confirm that by the  
addition of low strength 1-(2-chloro-5-pyridylmethyl)-2-  
nitroimino imidazolidine baits, spore concentrations  
irrespective of fungus species or strain, can be lowered by up  
to 4 powers of ten and more effective termite control will  
still be obtained than is obtained with soil treated with only  
water soaked baits.

TABLE 10

SYNERGISM WITH NITROGUANIDINE BAIT TREATMENTS AND A FUNGAL PATHOGEN

Rating Effect (1-9) Based on Average Termite Mortality after 5 days

No Spores of Metarhizium Anisopliae - MADA Strain (CBS No. 326)  
per gram of soil/Rating

% Nitroguanidine in dipping solution	No Spores of <u>Metarhizium Anisopliae</u> - MADA Strain (CBS No. 326) per gram of soil/Rating					
	0	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>5</sup>	10 <sup>7</sup>
0	9	9	9	9	9	5
0.00001	9	9	9	9	9	5
0.0001	9	9	9	9	8	(2)
0.001	8	8	6	(4)	(1)	(1)
0.01	7	6	4	(3)	(1)	(1)

○ Indicates strong expression of synergy

**EXAMPLE 11**

The procedure of Example 5 was repeated using the fungi Actinomucor sp., Paecilomyces farinosus, and Beauveria bassiana and the bacterium Serratia sp. The filter paper discs were soaked in either distilled water or a 0.001% solution of 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino) imidazolidine. The results observed over a 7 day period are reported in Table 11.

In Table 11, the rating system used and reported in the left column of each box was as follows:

- 10            -            Normal: no change as compared to untreated control.
  - +            Slight: some termites on surface or feeding and tunneling somewhat affected.
  - ++           Moderate: many termites on the surface with substantial reduction in feeding and tunneling; intoxication symptoms (sluggish, ataxial).
  - 15            +++           Severe: all termites on the surface with no feeding and tunneling; increasing intoxication symptoms.
  - 20            ++++           Lethal: 95-100% of termites dead or moribund.
- The numbers reported in the right column of each box in Table 11 are the % mortality.

25

30

Mo3729CIP

Table 11

Evaluation of Additional Fungus and Bacteria Isolates from Dead Termites for their Pathogenicity on *Reticulitermes Flavipes*

## Koch's Postulate

Inoculation of Soil	Treatment Filter Paper Disc	Behavioral Change and Mortality of Termites at Indicated Days After Treatment		
		2d	4d	6d-7d
None	None	0	0	0
None	0.001%	++	2 ++(+)	7 ++(+)
Actinomucor sp.	None	0	0	0
Actinomucor sp.	0.001%	++(+)	2 +++	14 ++++
Paecilomyces farinosus	None	0	0	0
Paecilomyces farinosus	0.001%	++	12 ++++	95 ++++

2086351

Table 11 Cont'd  
Behavioral Change and Mortality of Termites at Indicated Days After Treatment

Inoculation of Soil	Treatment Filter Paper Disc	2d	4d	6d-7d
Beauveria bassiana	None	-	1 +	16 ++ 18
Beauveria bassiana	0.001%	+(+)	1 +++	75 +++ 89
Serratia sp.	None	-	3 -	14 - 16
Serratia sp.	0.001%	+(+)	4 ++	53 +++ 68

1) Although the soil was autoclaved and the termites surface sterilized (by dipping into Chlorox solution), Metarhizium anisopliae probably from within the termites themselves, showed up as partially lethal for termites which had received 1-(2-chloro-5-pyridylmethyl)-2-(nitroimino)imidazolidine.

**EXAMPLE 12**

The procedure of Example 5 was repeated using filter paper disks dipped into various concentrations of several different nitromethylene compounds and two soil types. One type of soil was unsterile as taken from the field. The second type of soil was autoclaved. The concentrations of each nitromethylene and the results observed after 4 days are reported in Table 12.

The nitromethylenes used in this Example were as follows:

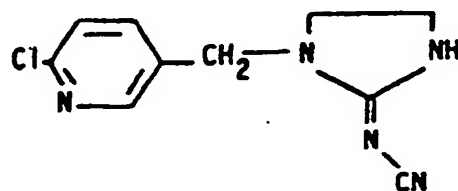
10

Compound

Formula

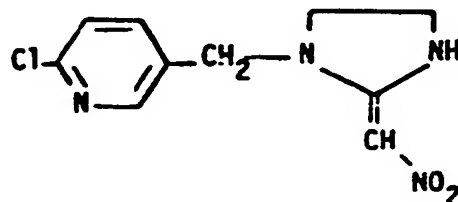
15

A



20

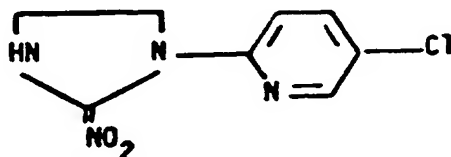
B



25

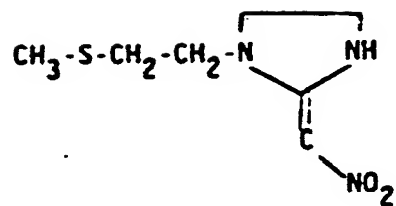
30

C



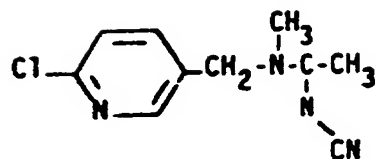
No3729C1P

D



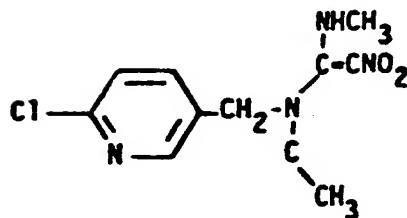
5

E



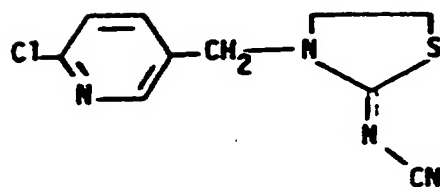
10

F



15

G



20

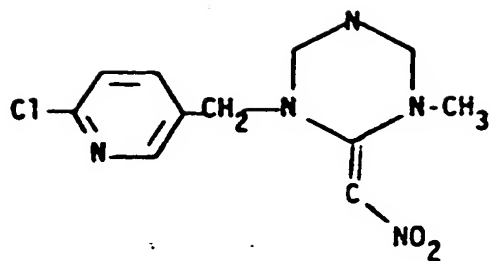
25

30

Mo3729C1P

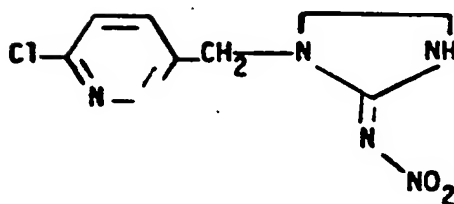


H



5

I



10

15

The degree of Intoxication is reported in Table 12 using the same rating system as was used in Table 11.

20

25

30

No3729CIP

2086351

Table 12

	Nitromethylene	% Nitromethylene	Degree Early	% Mortality after 4 days	
				Sterile Soil	Non-Sterile Soil
5	A	0.001	+	10 FF	100 FF
	B	0.1	++++	99 FF	100 FF
		0.01	++++	25 FF	100 FF
	C	0.1	+++	62 FF	100 FF
		0.01	+++	59 FF	100 FF
10		0.001	++	35 FF	100 FF
	D	0.1	++++	96 FF	100 FF
		0.01	+++	74 FF	100 FF
	E	0.1	++++	74 FF	100 FF
		0.01	+++	42 FF	100 FF
		0.001	+++	24 FF	100 FF
15	F	0.1	+++	22 FF	100 FF
		0.01	++	28 FF	100 FF
	G	0.1	++++	41 FF	100 FF
		0.01	+++	21 FF	100 FF
20	H	0.1	++	12 FF	100 FF
		0.01	+	6 FF	100 FF
		0.001	-	0	7 FF
	I	0.1	+++	18 FF	100 FF
		0.01	+++	13 FF	100 FF
		0.001	+++	20 FF	100 FF
25	Control				
	0.1% Solvent	-	-	0	4
	Water	-	-	0	0

1) Soil sterilization process was obviously incomplete.

30 "F" indicates spontaneous collapse of termite population due to occurrence of entomophagus fungi, mostly of the Metarhizium and Conidiobolus type. Each F represents one of 2 replicates.

Although the invention has been described in detail in the foregoing for the purpose of illustration, it is to be understood that such detail is solely for that purpose and that variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention except as it may be limited by the claims.

10

15

20

25

30

Mo3729CIP

WHAT IS CLAIMED IS:

1. A method for exterminating termites in which an effective amount of (a) at least one chemical selected from the group consisting of nitroguanidines, nitromethylenes, pyrazolines and pyrethroids and (b) an entomopathogenic fungus or bacterium is present at a site where termites have been observed or are suspected to be present.

2. The method of Claim 1 in which the chemical is a nitromethylene.

3. The method of Claim 1 in which the chemical is 1-(2-chloro-5-pyridylmethyl)-2-(nitromethylene) imidazolidine.

4. The method of Claim 3 in which the fungus is of the genus Actinomucor sp.

5. The method of Claim 3 in which the fungus is of the species Paecilomyces farinosus.

6. The method of Claim 3 in which the fungus is of the species Conidiobolus coronatus.

7. The method of Claim 3 in which the fungus is of the species Metarhizium anisopliae.

8. The method of Claim 2 in which the fungus is Actinomucor sp.

9. The method of Claim 2 in which the fungus is Paecilomyces farinosus.

10. The method of Claim 2 in which the fungus is Conidiobolus coronatus.

11. The method of Claim 2 in which the fungus is Metarhizium anisopliae.

12. The method of Claim 1 in which the fungus is Actinomucor sp.

13. The method of Claim 1 in which the fungus is Paecilomyces farinosus.

14. The method of Claim 1 in which the fungus is Conidiobolus coronatus.

15. The method of Claim 1 in which the fungus is Metarhizium anisopliae.

Mo3729CIP

16. A composition for controlling termites comprising (1) an effective amount of a chemical termiticide selected from the group consisting of nitroguanidines, nitromethylenes, pyrazolines and pyrethroids and (2) an entomopathogenic fungus or bacterium.

17. The composition of Claim 16 in which the chemical termiticide is a nitromethylene.

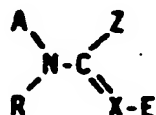
18. The composition of Claim 17 in which the fungus is Conidiobolus coronatus.

19. The composition of Claim 17 in which the fungus is Metarhizium anisopliae.

20. The composition of Claim 17 in which the fungus is Actinomyces sp.

21. The composition of Claim 17 in which the fungus is Paecilomyces farinosus.

22. A composition useful for extermination of termites which is composed of an effective amount (a) at least one compound represented by the formula



I

in which

R represents hydrogen, an acyl group, a substituted acyl group, an alkyl group, a substituted alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, or a substituted heterocyclic group;

A represents either (1) a monofunctional group selected from hydrogen, an acyl group, an alkyl group, an aryl group, a substituted acyl group, a substituted alkyl group, a substituted aryl group or (2) a bifunctional group which is connected to Z;

Mo3729CIP

- E represents an electron withdrawing group;  
X represents N or CH; and  
Z represents either (1) a monofunctional group selected from hydrogen, an acyl group, a substituted acyl group, an alkyl group, a substituted alkyl group, an aryl group, a substituted aryl group, a heterocyclic group, a substituted heterocyclic group, the group OR, NR<sub>2</sub>, SR or (2) a bifunctional group which is connected with either A or X;

and (b) an entomopathogenic fungus or bacterium.

23. The composition of Claim 22 in which in the compound represented by Formula I,

- Z represents NH, CH or NR'R" in which R' and R" each represents hydrogen or an alkyl group with at least one of R' and R" being an alkyl group,  
A represents an alkyl group,  
E represents CN or NO<sub>2</sub>,  
X represents N or CH and  
R represents a 5 or 6 membered heterocyclic ring optionally substituted with halogen or an alkyl group.

24. The composition of Claim 22 in which in the compound represented by Formula I,

- A and Z together represent a nitrogen containing 5 or 6 membered ring optionally substituted with a thioalkyl group or a sulfur containing 5 or 6 membered ring optionally substituted with a thioalkyl group,  
E represents CN or NO<sub>2</sub>,  
X represents N or CH and  
R represents a 5 or 6 membered heterocyclic ring optionally substituted with halogen or an alkyl group.

25. The composition of Claim 22 in which (b) is a fungus.

26. The composition of Claim 25 in which the fungus is Paecilomyces farinosus.

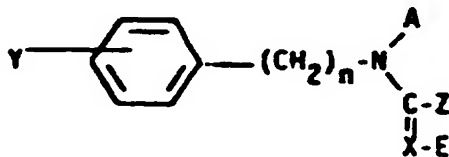
Mo3729CIP

27. The composition of Claim 25 in which the fungus is Paecilomyces farinosus.

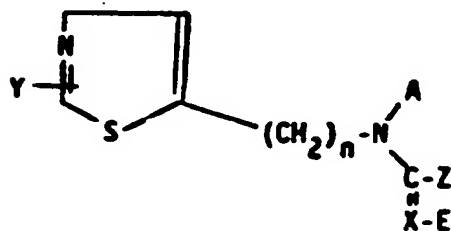
28. The composition of Claim 22 in which (b) is a bacterium.

29. A composition useful for extermination of termites which is composed of an effective amount of

(a) at least one compound represented by the formulae



or



in which

25     A     represents either (1) a monofunctional group selected from hydrogen, an acyl group, an alkyl group, an aryl group, a substituted acyl group, a substituted alkyl group, a substituted aryl group or (2) a bifunctional group which is connected to Z;

30     E     represents an electron withdrawing group;

      X     represents N or CH;

      Z     represents either (1) a monofunctional group selected from hydrogen, an acyl group, a substituted acyl group, an alkyl group, a substituted alkyl group, an

Mo3729CIP

aryl group, a substituted aaryl group, a heterocyclic group, a substituted heterocyclic group, the group OR, NR<sub>2</sub>, SR or (2) a bifunctional group which is connected with either A or X;

5           Y   represents an alkyl group having from 1 to 4 carbon atoms, an alkoxy group having from 1 to 6 carbon atoms, an alkylthio group having from 1 to 4 carbon atoms, a haloalkyl group having from 1 to 4 carbon atoms, a hydroxyl group, halogen, an amino group, an  
10           alkylamino group, a carboxyl group, an alkoxy carbonyl group, a sulfo group, an alkyl sulfonyl group or an aaryl sulfonyl group and

n   represents 1 or 2

and

15   (b) an entomopathogenic fungus or bacterium.

30. The composition of Claim 29 in which (b) is an entomopathogenic fungus.

31. The composition of Claim 30 in which the fungus is Actinomucor sp.

20   32. The composition of Claim 30 in which the fungus is Paecilomyces farinosus.

33 The composition of Claim 29 in which (b) is a bacterium.

25

30